British Journal of Pharmacology (1999) 127, 1846–1850
http://www.stockton-press.co.uk/bjp

Effect of Na^+/Ca^{2+} exchange inhibitor, KB-R7943 on ouabain-induced arrhythmias in guinea-pigs

*,[1]Tomokazu Watano, [2]Yoshimitsu Harada, [2]Kengo Harada & [2]Noriyasu Nishimura

[1]Department of Pharmacology, Fukushima Medical University, School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan and [2]Pharmacology Group, R&D Laboratories, Research & Development Division, Nippon Organon K.K., 5-90, Tomobuchi-cho 1-chome, Miyakojima-ku, Osaka 534-8666, Japan

1 We investigated protective effects of KB-R7943, a Na^+/Ca^{2+} exchange (NCX) inhibitor, on ouabain-induced tonotropy and arrhythmias in isolated whole atria and ouabain-induced changes in electrocardiogram (ECG) in the guinea-pig.

2 KB-R7943 (10 and 30 μM) suppressed the tonotropic effect of ouabain, and prolonged the onset time of extra-systole induced by ouabain in isolated atria.

3 The intravenous injection of KB-R7943 (1 and 3 mg kg^{-1}) significantly increased the doses of ouabain required to induce ventricular premature beats (VPB), ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest (CA) in anaesthetized guinea-pigs.

4 Lidocaine (Na^+channel inhibitor) and R56865 (Na^+ and Ca^{2+} overload inhibitor) also suppressed the ouabain-induced tonotropic effect and extra-systole in isolated atria, but Hoe-694 (Na^+/H^+ exchange inhibitor) or diltiazem (Ca^{2+} channel inhibitor) did not affect them.

5 Lidocaine also increased the doses of ouabain required to induce VPB, VT, VF and CA in anaesthetized guinea-pigs.

6 From these results, we conclude that KB-R7943 suppresses ouabain-induced arrhythmias through inhibition of the reverse-mode NCX.

Keywords: Na^+/Ca^{2+} exchange (NCX); KB-R7943; lidocaine; ouabain-induced arrhythmias; heart; positive tonotropic effect

Abbreviations: CA, cardiac arrest; DCB, 3′,4′-dichlorobenzamil; DMSO, dimethylsulphoxide; ECG, electrocardiogram; KB-R7943, 2-[2-[4-nitrobenzyloxyl]phenyl]ethyl)isothiourea methanesulphonate; NCX, Na^+/Ca^{2+} exchange; TI, transient inward current; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia

Introduction

Cardiac glycosides have been used as therapeutic agents to increase the force of cardiac contraction. However, these agents, such as ouabain, often induce cardiac arrhythmia accompanied by an increase in resting tension (Kass *et al*., 1978; Khatter *et al*., 1986). This symptom is called a triggered pacemaker type arrhythmia. Ouabain inhibits plasma membrane Na^+/K^+-ATPase, and rises intracellular Na^+ concentration. The rise in intracellular Na^+ concentration causes intracellular Ca^{2+} overload due to activation of sarcolemmal Na^+/Ca^{2+} exchange (NCX). The Ca^{2+} overload in turn induces oscillatory Ca^{2+} release from sarcoplasmic reticulum and oscillatory fluctuation in resting potential. An ionic current associated with this Ca^{2+} oscillation has been named a transient inward current (TI). It has been reported that about 75% of TI is attributed to an ionic current generated by NCX while the remaining current is mediated through non-specific cation channels (Lederer & Tsien, 1976; Kimura 1987).

The oscillatory Ca^{2+} rise by TI induces the enhancement of systolic (positive inotropic effect), and diastolic tension (positive tonotropic effect) and the extra-beatings (arrhythmias).

The potent and selective inhibitory effect of KB-R7943 (2-[2-[4-nitrobenzyloxyl]phenyl]ethyl)isothiourea methanesulphonate) on NCX has been reported (Watano *et al*., 1996; Iwamoto *et al*., 1996). KB-R7943 inhibited outward NCX current (reverse-mode of NCX) more potently than inward NCX current (forward-mode of NCX) in guinea-pig cardiac ventricular cells. This compound also inhibited ionic currents mediated through voltage-gated Na^+ and Ca^{2+} channels and inward rectifier K^+ channels in these cells. KB-R7943 also inhibited $[Na^+]_i$-dependent $[Ca^{2+}]_i$ increase which is mediated through the reverse-mode of NCX in cardiomyocytes, smooth muscle cells and NCX1-transfected fibroblasts (Iwamoto *et al*., 1996). In contrast, 3′,4′-dichlorobenzamil (DCB) inhibited the inward (forward-mode) NCX current more potently than outward (reverse-mode) NCX current (Watano *et al*., 1996).

In the present study, we examined the protective effects of KB-R7943, the above-mentioned NCX inhibitor, on ouabain-induced changes in contraction and arrhythmias in whole atria isolated from guinea-pigs. And these protective effects were compared with those of DCB (forward-mode NCX inhibitor), lidocaine (Na^+ channel inhibitor, class I antiarrhythmic agent), diltiazem (Ca^{2+} channel inhibitor, class IV antiarrhythmic agent), Hoe-694 (Na^+/H^+ exchange inhibitor) and R56865 (Na^+ and Ca^{2+} overload inhibitor). We also examined the protective effects of KB-R7943 on ouabain-induced arrhythmias in anaesthetized guinea-pigs and compared these effects with those of lidocaine.

*Author for correspondence; E-mail: watano@cc.fmu.ac.jp

Methods

Isolated whole atria

The whole atria were isolated from the hearts of male Hartley guinea-pigs weighing 300–400 g and bathed in Tyrode solution containing (in mM): NaCl 137, KCl 6, $CaCl_2$ 1.82, $MgCl_2$ 1.05, NaH_2PO_4 0.417, $NaHCO_3$ 11.5 and glucose 5.5. The Tyrode solution was maintained at 30°C and continuously aerated with 95% O_2–5% CO_2 (pH 7.2–7.4 under these conditions). One end of the muscle was attached to a rigid support and the other end was attached to a force/displacement transducer (TB-611T, Nihon-Kohden, Japan) *via* a silk suture in the 30 ml bath. Each tissue was placed under 0.5 g tension. Following stabilization of the spontaneous beating of preparations for about 45 min with replacing the solution every 15 min, drugs were added. Ouabain (3 μM) was added 45 min after the addition of the drugs. The contraction of atria was recorded continuously for 90 min after the addition of ouabain (see Figure 1a). Beating rates were counted from the signals of the contraction measured.

Anaesthetized guinea-pigs

Male Hartley guinea-pigs weighing 340–710 g were anaesthetized by intraperitoneal injection of pentobarbital sodium (50 mg kg^{-1}). Body temperature was maintained at about 37°C by a water blanket throughout the experiment. Artificial respiration was maintained at a rate of 55 r.p.m. and a volume was adjusted at 10 ml kg^{-1} by a rodent respirator (SN-480-7, Sinano, Japan). The right common carotid artery was cannulated with polyethylene tubing and connected to a transducer (TP-400T, Nihon-Kohden, Japan). Blood pressure was recorded by an amplifier (AP-641G, Nihon-Kohden), and instantaneous heart rate was counted by a heart rate counter (AT-601G, Nihon-Kohden) from the pulses of the blood pressure. A polyethylene tubing for the infusion of ouabain and test drugs was cannulated into the left jugular vein. Limb lead II electrocardiogram (ECG) was recorded on a pen-writing recorder polygraph (RM-6000, Nihon-Khoden). Following a stabilization period, test drugs were administered. Five minutes after the administration, a solution containing ouabain (100 μg ml^{-1}) was continuously infused at the rate of 100 μl min^{-1} kg^{-1} using a micro-infusion pump (CFV-2100, Nihon-Kohden). The amounts of ouabain required per kg body weight for the onset time of ventricular premature beats (VPB), ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest (CA) were adopted as indexes for arrhythmias (see Figure 5).

Solutions and drugs

KB-R7943 (2-[2-[4-(4-Nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulphonate, Kanebo, Osaka, Japan), DCB (3′,4′-dichlorobenzamil, Kanebo), lidocaine hydrochloride (Sigma, U.S.A.), Hoe-694 (Kanebo) and R56865 (Kanebo) were first dissolved in dimethylsulphoxide (DMSO). Diltiazem (Sigma) and ouabain (Sigma) was first dissolved in water. These solutions were added to the bath solution (for isolated atria) or diluted with physiological saline (for anaesthetized guinea-pigs). The final concentration of DMSO was 0.1% or less. For control experiments, 0.1% DMSO alone was applied.

Statistical analysis

All values were expressed as mean ± s.e.mean. Statistical analysis was performed by Dunnett's multiple-range test. Significance was evaluated when the probability value was less than 0.05.

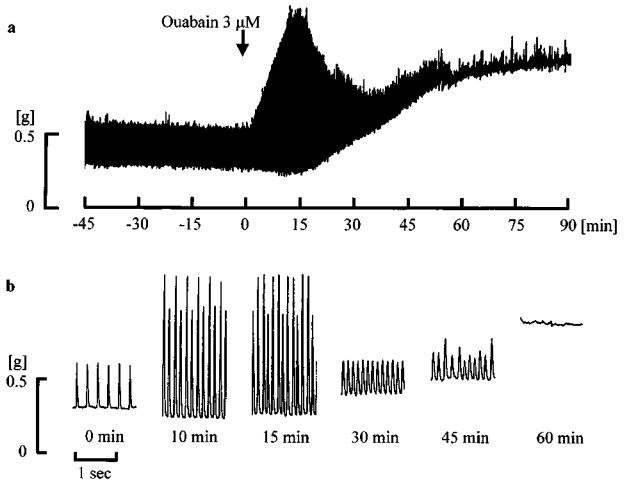

Figure 1 Original recording of the force of contraction of spontaneously beating guinea-pig isolated whole atria treated by 3 μM ouabain. (a) Continuous recording of contraction. (b) Time course of the contraction shown as expanded traces.

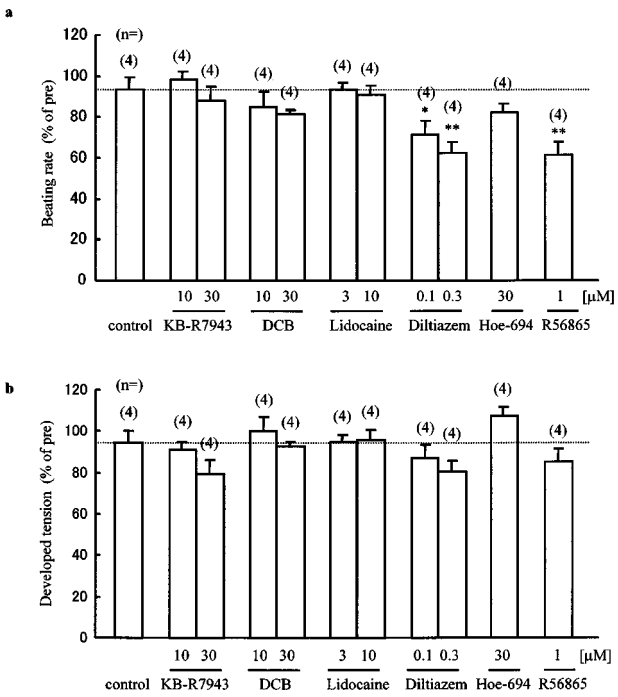

Figure 2 Effects of KB-R7943 and other compounds on the contraction of spontaneously beating guinea-pig isolated atria. (a) Effects on beating rates. KB-R7943 and other compounds were treated for 45 min before the measurement. Data are shown as percentages of the beating rates before the treatment. (b) Effects on developed tension. Data are shown as percentages of the developed tension before the treatment. $^*P<0.05$, $^{**}P<0.01$ vs control (Dunnett's multiple-range test). Each value is the mean ± s.e.mean of at least four experiments.

Results

Effect of KB-R7943 on ouabain toxicity in whole atria

Figure 1 shows typical recording for spontaneously beating of isolated whole atria. The addition of 3 μM ouabain produced the enhancement of systolic tension (positive inotropic effect; Figure 1a) and extra beatings (arrhythmias; Figure 1b). The positive inotropic effect and arrhythmias reached their maxim about 10 min after the addition of ouabain. The enhancement of diastolic tension (positive tonotropic effect) then appeared about 15 min after addition of ouabain and continued for about 60 min (Figure 1a). In addition to these inotropic and tonotropic effects, arrhythmias was observed about 10 min after the addition of ouabain and thereafter (Figure 1b). Spontaneous beatings were gradually decreased and arrested 60 min after the addition of ouabain (Figure 1b).

We first examined the effects of KB-R7943 and other agents on basal beating rates and developed tension in the absence of ouabain. These drugs were applied 45 min after the preparation of spontaneously beating atria in this case. KB-R7943 (10 or 30 μM) did not significantly affect beating rates (Figure 2a) or developed tension (Figure 2b). DCB (10 or 30 μM), lidocaine (3 or 10 μM) or Hoe-694 (30 μM) had also no effects on beating rates (Figure 2a) or developed tension (Figure 2b). Diltiazem (0.1 or 0.3 μM) and R56865 at 1 μM decreased beating rates (Figure 2a), but did not significantly affect developed tension (Figure 2b).

We next examined the effects of KB-R7943 and other agents on the positive inotropic and tonotropic effects of ouabain and the arrhythmias induced by ouabain. Figure 3 shows typical recording for ouabain (3 μM)-induced contraction change of isolated whole atria in the absence (Figure 3a) or presence (Figure 3b) of KB-R7943 (30 μM). Pre-treatment with KB-R7943 (10 or 30 μM) did not significantly affect the positive inotropic effect (Figures 3b and 4a). However, with this pre-treatment with KB-R7943 the positive tonotropic effect was reduced (Figures 3b and 4b) and the onset time of arrhythmia was prolonged (Figure 4c). Lidocaine (3 or 10 μM) did not significantly affect the positive inotropic effect (Figure 4a), but it reduced the positive tonotropic effect (Figure 4b) and prolonged the onset time of arrhythmia (Figure 4c). Hoe-694 (30 μM) did not significantly affect the positive inotropic effect (Figure 4a), the positive tonotropic effect

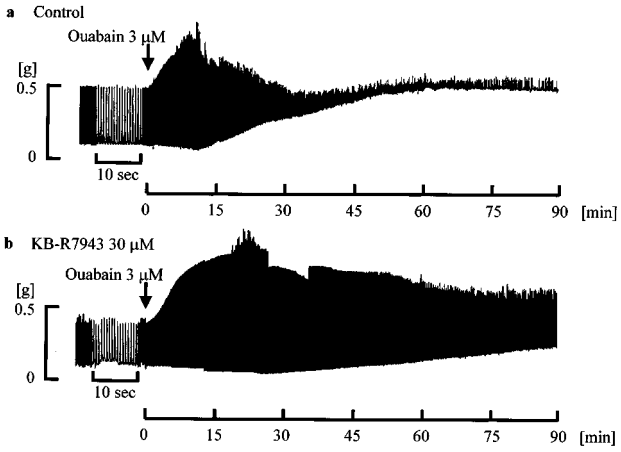

Figure 3 Effects of KB-R7943 on the contraction of spontaneously beating guinea-pig isolated whole atria treated by 3 μM ouabain. (a) The contraction change treated by 3 μM ouabain in the absence of KB-R7943. (b) Effect of KB-R7943 (30 μM) on the contraction change.

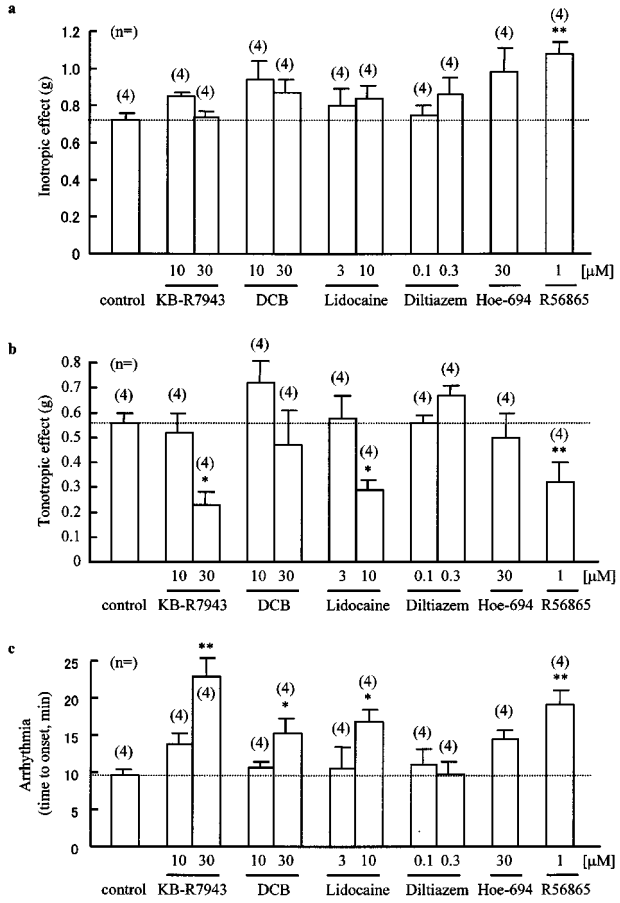

Figure 4 Effects of KB-R7943 and other compounds on positive inotropic and tonotropic effects of ouabain and time to the onset of arrhythmia induced by ouabain in guinea-pig isolated atria. The concentration of ouabain was 3 μM. (a) Effects on the positive inotropic effect. (b) Effects on the positive tonotropic effect. (c) Effects on time to the onset of the arrhythmia. $*P<0.05$, $**P<0.01$ vs control (Dunnett's multiple-range test). Each value is the mean$\pm$s.e.mean of at least four experiments.

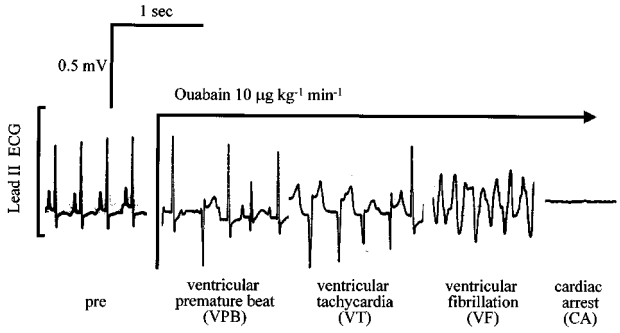

Figure 5 Typical ECG changes indicating arrhythmia indexes in anaesthetized guinea-pigs. ECG before the application of ouabain (Pre) and the changes observed after the application of ouabain, namely ventricular premature beats (VPB), ventricular fibrillation (VF), ventricular tachycardia (VT) and cardiac arrest (CA) induced by ouabain are shown.

(Figure 4b) or the onset time of arrhythmia (Figure 4c). DCB (10 or 30 μM) did not significantly affect the positive inotropic effect (Figure 4a) or the positive tonotropic effect (Figure 4b), while it prolonged the onset time of arrhythmia at 30 μM (Figure 4c). Diltiazem (0.1 or 0.3 μM) did not significantly affect the positive inotropic effect (Figure 3a), the positive tonotropic effect (Figure 4b) or the onset time of the arrhythmia (Figure 4c). R56865 at 1 μM enhanced the positive inotropic effect (Figure 4a), reduced the positive tonotropic effect (Figure 4b) and prolonged the onset time of arrhythmia (Figure 4c).

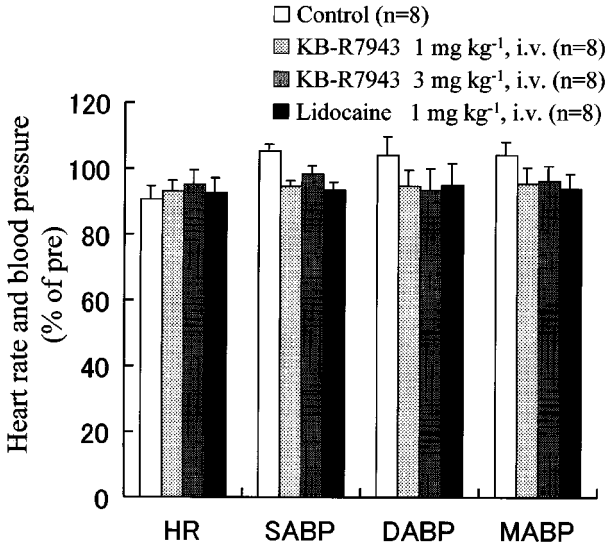

Figure 6 Effects of KB-R7943 and lidocaine on basal heart rate (HR), systolic arterial blood pressure (SABP), diastolic arterial blood pressure (DABP) and mean arterial blood pressure (MABP) in anaesthetized guinea-pigs. Data are shown as percentages of the values before the treatment. Each column indicates the mean$\pm$ s.e.mean of at least eight experiments.

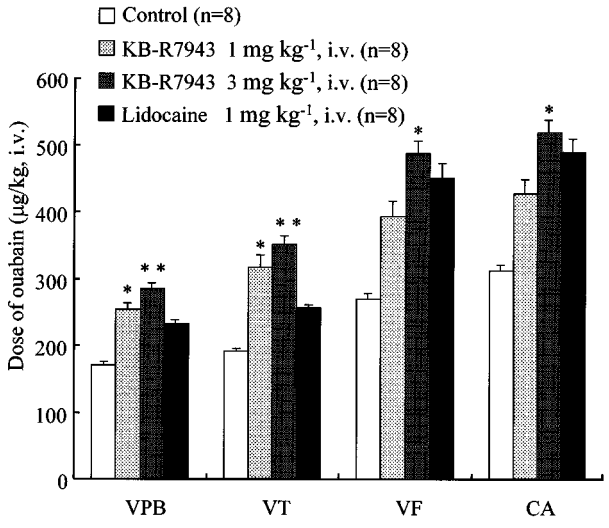

Figure 7 Effect of KB-R7943 and lidocaine on ventricular premature beats (VPB), ventricular fibrillation (VF), ventricular tachycardia (VT) and cardiac arrest (CA) induced by ouabain in anaesthetized guinea-pigs. The data are shown as the doses of ouabain required inducing the arrhythmia indexes. $P<0.05$, $^{**}P<0.01$ vs control (Dunnett's multiple-range test). Each value is the mean$\pm$s.e.mean of at least eight experiments.

Effect of KB-R7943 on ouabain-induced ECG change

Basal heart rate (HR), systolic arterial blood pressure (SABP), diastolic arterial blood pressure (DABP) and the mean arterial blood pressure (MABP) in anaesthetized guinea-pigs were not significantly different among experimental groups. The intravenous injection of KB-R7943 (1 or 3 mg kg^{-1}) or lidocaine (1 mg kg^{-1}) did not affect these perimeters measured 5 min after the injection (Figure 6).

Figure 5 shows typical recording for ECG before the intravenous infusion of ouabain and ventricular premature beats (VPB), ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest (CA) observed after the infusion of ouabain. Figure 7 compares the doses of ouabain required to provoke VPB, VT, VF and CA. Ouabain induced cardiac arrhythmias (VPB, VT and VF) and CA in a dose range between 170 and 310 μg kg^{-1}. The intravenous injection of KB-R7943 (1 and 3 mg kg^{-1}) significantly increased the doses of ouabain required to induce arrhythmias (VPB, VT and VF) and CA (Figure 7). Lidocaine (1 mg kg^{-1}) also tended to increase the doses of ouabain required to induce arrhythmias (VPB, VT and VF) and CA (Figure 7).

Discussion

KB-R7943 significantly reduced the positive tonotropic effect of ouabain and inhibited arrhythmias (extra-systole) induced by ouabain in whole atria isolated from guinea-pigs. DCB did not reduce the positive tonotropic effect, but inhibited the arrhythmias. We have already shown that KB-R7943 inhibits the reverse-mode of NCX more potently than the forward-mode of NCX while DCB inhibits the forward-mode of NCX more potently than the reverse-mode of NCX (Watano *et al*., 1996; Iwamoto *et al*., 1996). High doses of ouabain often induce triggered activity type cardiac arrhythmias (Kass *et al*., 1978). Ouabain inhibits plasma membrane Na^+/K^+-ATPases, leading a rise in intracellular Na^+ levels. The rise in intracellular Na^+ levels causes intracellular Ca^{2+} overload through the reverse-mode of NCX (Khatter *et al*., 1989). This overloaded Ca^{2+} is taken up by sarcoplasmic reticulum, but, after the saturation of sarcoplasmic reticulum, Ca^{2+} oscillation is evoked by Ca^{2+} release from sarcoplasmic reticulum. The Ca^{2+} oscillation then induces Na^+ influx through the forward-mode of NCX and non-selective cation channels. These Na^+ influx forms an ionic current component identified as TI (Lederer & Tsien, 1976; Kimura *et al*., 1987). TI induces oscillatory depolarization, and, when the depolarization reaches a threshold, voltage-gated Na^+ channels are activated, resulting the generation of an action potential. This action potential does not serve as a regular beat, but an extra systole, and causes a cardiac arrhythmias (Kass *et al*., 1978; Wier *et al*., 1984). From these processes proposed, it is expected that NCX inhibitors block the ouabain-induced arrhythmias. In fact, in the present study, KB-R7943 and DCB inhibited the ouabain-induced arrhythmias in isolated guinea-pig atria. However, it is noted that, in addition to the inhibition of NCX, high doses of KB-R7943 inhibit voltage-gated Na^+ channels and Ca^{2+} channels (Watano *et al*., 1996), and high doses of DCB inhibit voltage-gated Na^+ channels and Na^+/H^+ exchange (Murata *et al*., 1995). To determine which effects of KB-R7943 or DCB play essential roles in the inhibition of the ouabain-induced arrhythmias, we also examined the effects of a Na^+ channel inhibitor, a Ca^{2+} channel inhibitor and a Na^+/H^+ exchange inhibitor in the present study. Lidocaine (Na^+ channel inhibitor) and R56865 (Na^+ and Ca^{2+} overload inhibitor) also inhibited the ouabain-induced arrhythmias. These protective

effects of the Na^+ channel inhibitor and the Na^+ and Ca^{2+} overload inhibitor support the proposed processes for the ouabain-induced arrhythmias described above. The results also suggest that, in addition to activity as an NCX inhibitor, the inhibition by KB-R7943 or DCB of voltage-gated Na^+ channels partly contribute to the inhibition by these compounds of the inhibition of the ouabain-induced arrhythmias. On the other hand, Hoe-694 (Na^+/H^+ exchange inhibitor) or diltiazem (Ca^{2+} channel inhibitor) did not affect the ouabain-induced arrhythmias. Consequently, Na^+/H^+ exchange or voltage-gated Ca^{2+} channels may not have important roles in the ouabain-induced toxicity in guinea-pig isolated atria.

Ouabain induced a positive tonotropic effect (rising of diastolic tension; Figure 1a). As described above, the inhibition by ouabain of plasma membrane Na^+/K^+-ATPases results in a rise in intracellular Na^+ levels, and the rise in intracellular Na^+ levels causes intracellular Ca^{2+} overload through the reverse-mode of NCX. In accord with this speculation, KB-R7943, a reverse-mode NCX inhibitor inhibited the positive tonotropic effect, but DCB, a forward-mode NCX inhibitor, did not significantly affect the positive tonotropic effect in the present study.

None of the agents examined in the present study reduced the positive inotropic effect of ouabain. The positive inotropic effect of ouabain can also be explained by the inhibition of plasma membrane Na^+/K^+-ATPases, which finally leads to intracellular Ca^{2+} overload. In contrast to this explanation, it has been reported that the positive inotropic effect of ouabain is attributed to activation of phospholipase C and an increase in diacylglycerol levels, which causes activation of protein kinase C (Gotoh *et al*., 1993). The mechanism underlying the ouabain-induced positive inotropic effect may be different from that underlying the positive tonotropic effect.

We found that KB-R7943 inhibits ouabain-induced cardiac arrhythmias and cardiac arrest in anaesthetized guinea-pigs. It has been hypothesized that cardiac ischaemia caused by intracellular acidosis is attributed to anaerobic metabolism (Steenbergen *et al*., 1977; Allen *et al*., 1993) and ATP hydrolysis (Dennis *et al*., 1991). These changes result in activation of Na^+/H^+ exchange system and cause Na^+ influx and H^+ efflux (Frelin *et al*., 1984; Poole-Wilson, 1989). The Na^+ influx increases intracellular Na^+ concentration, and this in turn reveals either the reduction of Ca^{2+} efflux *via* the forward-mode of NCX or the induction of Ca^{2+} influx through the reverse-mode of NCX, depending on Na^+ gradient across plasma membrane and membrane potential. The Ca^{2+} overload caused by these mechanisms may induce cardiac arrhythmias (Coetzee & Opie, 1987). Based on these mechanisms, it is expected that cardiac ischaemia and reperfusion injury are prevented by inhibiting the Ca^{2+} influx *via* NCX. The protective effects of KB-R7943 on ischaemia/reperfusion-induced injury in isolated rat perfused hearts (Nakamura *et al*., 1998) may be explained by such NCX-based mechanisms. A possibility that the inhibition by KB-R7943 of voltage-gated Na^+ channels contributes to the protection of arrhythmias, however, cannot be excluded because lidocaine also tended to inhibit the ouabain-induced cardiac arrhythmias and arrest in the present study.

In conclusion, KB-R7943, an NCX inhibitor, protected ouabain-induced arrhythmias both in isolated atria and in anaesthetized guinea-pigs. This finding may raise a possibility that the inhibition of NCX provides a novel point of view to the development of antiarrhythmic drugs.

The authors would like to thank Professor Junko Kimura, Department of Pharmacology, Fukushima Medical University, School of medicine and Dr Ken Nakazawa, Division of Pharmacology, National Institute of Health Sciences for helpful discussion and critical reading.

References

ALLEN, D.G., CAIRNS, S.P., TURVEY, S.E. & LEE, J.A. (1993). Intracellular calcium and myocardial function during ischemia. *Adv. Exp. Med. Biol.,* **346,** 19–29.

COETZEE, W.A. & OPIE, L.H. (1987). Effects of components of ischemia and metabolic inhibition on delayed afterdepolarizations in guinea pig papillary muscle. *Circ. Res.,* **61,** 157–165.

DENNIS, S.C., GEVERS, W. & OPIE, L.H. (1991). Protons in ischemia: where do they come from; where do they go to? [editorial]. *J. Mol. Cell Cardiol.,* **23,** 1077–1086.

FRELIN, C., VIGNE, P. & LAZDUNSKI, M. (1984). The role of the Na^+/H^+ exchange system in cardiac cells in relation to the control of the internal Na^+ concentration. A molecular basis for the antagonistic effect of ouabain and amiloride on the heart. *J. Biol. Chem.,* **259,** 8880–8885.

GOTOH, H., KAMIYAMA, A., SHIBAYAMA, R., SAWADA, M. & KASHIMOTO, T. (1993). Involvement of phosphoinositide turnover in ouabain inotropism. *Biochem. Biophys. Res. Commun.,* **194,** 72–78.

IWAMOTO, T., WATANO, T. & SHIGEKAWA, M. (1996). A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. *J. Biol. Chem.,* **271,** 22391–22397.

KASS, R.S., LEDERER, W.J., TSIEN, R.W. & WEINGART, R. (1978). Role of calcium ions in transient inward currents and after-contractions induced by strophanthidin in cardiac Purkinje fibers. *J. Physiol. (Lond.),* **281,** 187–208.

KHATTER, J.C., AGBANYO, M., HOESCHEN, R.J., NAVARATNAM, S. & BAINS, R. (1986). Digitalis-induced mechanical toxicity: protection by slow Ca^{++} channel blockers. *J. Pharmacol. Exp. Ther.,* **239,** 206–210.

KHATTER, J.C., AGBANYO, M., NAVARATNAM, S., NERO, B. & HOESCHEN, R.J. (1989). Digitalis cardiotoxicity: cellular calcium overload a possible mechanism. *Basic. Res. Cardiol.,* **84,** 553–563.

KIMURA, J., MIYAMAE, S. & NOMA, A. (1987). Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. *J. Physiol. (Lond.),* **384,** 199–222.

LEDERER, W.J. & TSIEN, R.W. (1976). Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. *J. Physiol. (Lond.),* **263,** 73–100.

MURATA, Y., HARADA, K., NAKAJIMA, F., MARUO, J. & MORITA, T. (1995). Non-selective effects of amiloride and its analogues on ion transport systems and their cytotoxicities in cardiac myocytes. *Jpn. J. Pharmacol.,* **68,** 279–285.

NAKAMURA, A., HARADA, K., SUGIMOTO, H., NAKAJIMA, F. & NISHIMURA, N. (1998). [Effects of KB-R7943, a novel Na^+/Ca^{2+} exchange inhibitor, on myocardial ischemia/reperfusion injury]. *Folia. Pharmacol. Jpn.,* **111,** 105–115.

POOLE-WILSON, P.A. (1989). Regulation of intracellular pH in the myocardium; relevance to pathology. *Mol. Cell Biochem.,* **89,** 151–155.

STEENBERGEN, C., DELEEUW, G., RICH, T. & WILLIAMSON, J.R. (1977). Effects of acidosis and ischemia on contractility and intracellular pH of rat heart. *Circ. Res.,* **41,** 849–858.

WATANO, T., KIMURA, J., MORITA, T. & NAKANISHI, H. (1996). A novel antagonist, No. 7943, of the Na^+/Ca^{2+} exchange current in guinea-pig cardiac ventricular cells. *Br. J. Pharmacol.,* **119,** 555–563.

WIER, W.G. & HESS, P. (1984). Excitation-contraction coupling in cardiac Purkinje fibers. Effects of cardiotonic steroids on the intracellular $[Ca^{2+}]$ transient, membrane potential, and contraction. *J. Gen. Physiol.,* **83,** 395–415.

(Received December 12, 1998
Revised March 11, 1999
Accepted May 27, 1999)